The effect of cereal type and micronisation on the concentration of lactic acid in the production of fermented liquid feed for pigs.

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Introduction

The use of fermented liquid feeds (FLF) is gaining popularity in Europe as a means of improving the gut health of pigs and improving the stability and safety of liquid feed in *ad libitum* feeding systems. With modern liquid feeding systems it is often advantageous to ferment the cereal component of the diet and use this as a base to which other components are added to formulate a range of diets for the whole unit. The exception to this may be diets for newly weaned pigs where it is normal (in the UK) for processed cereals to be used.

Liquid feed provides an ideal medium for the growth of a range of micro-organisms which compete for nutrients. If left alone spontaneous lactic acid fermentation normally occurs and as lactic acid concentrations increase pH is reduced and competing organisms die out or suffer a marked reduction in growth rates, a process similar to that which occurs in forage silages. High lactic acid concentrations and low pH contribute to the stability of the feed and prevent the growth of undesirable organisms such as *Salmonella* spp. In addition to these beneficits in feed safety FLF has beneficial effects on gut health. Van Winsen (2001) showed that the numbers of *Enterobacteriaceae* in the contents of the stomach, ileum, cecum, colon, and rectum of pigs fed fermented feed were significantly lower than the contents of the stomach, ileum, caecum, colon, and rectum of pigs fed dry feed.

Salmonella can persist in FLF containing less that 75 mmol/L lactic acid (Beal *et al.* 2002). If the goal of fermentation is to achieve lactic acid concentrations greater than 75 mmol/L at the point of delivery to the pig, this in essence means achieving > ca 110 mmol/L lactic acid in a fermented cereal base. Whilst these levels of lactic acid are rarely achievable in spontaneously fermented cereals (Beal *et al.* 2005), they can be readily achieved with the use of starter cultures. However, most commercial starter cultures have been developed for use in dairy products or for the production of silage. None have been developed for the specific purpose of fermenting liquid feed for pigs. In this study the effect of substrate in the form of four different cereals, barley, wheat, oats and maize, on the production of lactic acid by four different starter cultures was investigated. In the UK heat treated cereals are commonly used in diets for newly weaned pigs. Therefore, in addition to the unprocessed cereals the effect of micronization on lactic acid production was investigated.

Methods

The study was conducted as a three factor factorial, Factor 1 was cereal processing (raw or micronised. Factor 2 was cereal type (wheat, barley, oats or maize) and Factor 3 was lactic acid bacteria inoculant (*Pediococcus acidilactici* (PA), *Lactobacillus farciminis* (LF), *Lactobacillus plantarum* (LP) or *Lactobacillus salivarius* (LS)). Raw cereals were ground in a disc mill (Skiold2500 Danagri Bridgnorth UK) using a setting of 0 and along with the micronised cereals were sterilized by irradiation (25kG □ radiation from ^{60}Co). Sterile liquid cereals were prepared by mixing 100g cereal with 250 ml sterile distilled water. Triplicate samples of each cereal were inoculated with a 0.01% (v/v) of a 24h broth culture (de Mann rogosa Sharpe Broth (Oxoid Basingstoke UK)) of each LAB and incubated at 30°C for 30h. Samples taken at 0, 4, 8, 24 and 30h after the commencement of fermentation were analysed for lactic acid by HPLC using the method of Niven *et al* (2004).

The results were analysed by analysis of variance using a general linear model (Minitab release 14)

Results

In all liquid grains lactic acid levels started to increase between the 4 h and 8 h sampling point and thereafter increased linearly for a further 16h after which the rate of lactic acid production decreased in some samples. All fermentations followed a similar pattern, data for *Lb salivarius* (LS) is shown in Figure 1.

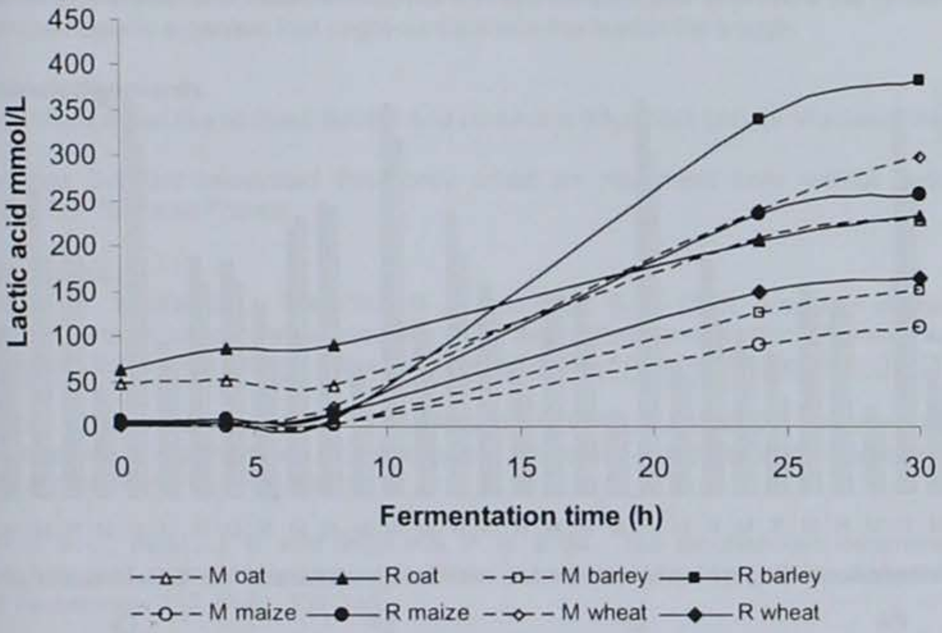

Figure 1 Lactic acid production in raw (R) or micronised (M) liquid cereals fermented for 30 h at 30°C with *Lb salivarius*.

The lactic acid concentration in the cereals prior to fermentation was variable and ranged from 2.5 mmol L^{-1} in wheat to 63 mmol L^{-1} in oats. Therefore the efficacy of fermentation was evaluated by taking the rate of lactic acid production between 8 and 24h fermentation (Table 1) rather than the total lactic acid produced. There was a significantly higher ($P > 0.001$) rate of lactic acid production in raw barley with all LAB than there was from any other cereal.

Table 1 Rate of Lactic acid produced (mmol L^{-1} h^{-1}) in raw (R) or micronised (M) liquid cereal (1 cereal:2.5 water) fermented with *P. acidilactici* (PA), *Lb. farciminis* (LF), *Lb. plantarum* (LP) or *Lb. salivarius* (LS)

Fermenting organism	Wheat		Barley		Oats		Maize	
	R	M	R	M	R	M	R	M
PA	5.28^{a1}	9.75^{b1}	18.48^{1}	5.24^{a1}	6.44^{a12}	1.47	9.47^{b1}	4.28^{a1}
LF	6.11^{a1}	10.85^{b1}	20.48^{12}	6.39^{a12}	5.61^{a1}	5.82^{a1}	9.01^{b1}	4.14^{a1}
LP	8.64^{a2}	15.72^{b2}	21.33^{2}	9.55^{a3}	8.65^{a2}	7.82^{a1}	16.97^{b2}	6.14^{a1}
LS	8.51^{ac2}	13.64^{d2}	20.79^{12}	7.77^{abc23}	7.23^{ab12}	10.09^{c}	13.95^{d2}	5.55^{b1}

^{abcd} means in the same row with the same superscript are not significantly different ($P > 0.05$)
^{123} means in the same column are not significantly different ($P > 0.05$)
s.e.m = 0.4512

For barley and maize all four lactic acid bacteria produced significantly more lactic acid from the raw cereal compared with the micronised cereal. The opposite was seen with wheat where the rate of lactic acid production was significantly higher ($P<0.001$) in micronised wheat compared with raw wheat for all four lactic acid bacteria. In oats the picture was more variable with *P. acidilactici* the rate of lactic acid production was significantly higher ($P <0.001$) in raw oats, whereas with *Lb. salivarius* the rate of production was significantly higher ($P<0.05$) in micronised oats. There was no significant difference ($P>0.05$) in the rate of lactic acid produced in raw or micronised oats fermented with *Lb. farciminis* or *Lb. plantarum*.

The goal of fermentation was to achieve 110 mmol L^{-1} lactic acid. After 24 h this was achieved in the most of the raw cereals, the exceptions being raw wheat fermented with *P. acidilactici* and *Lb. farciminis*._ After 30 h fermentation the all raw cereals contained at least 110 mmol L^{-1} lactic acid (Figure 2).

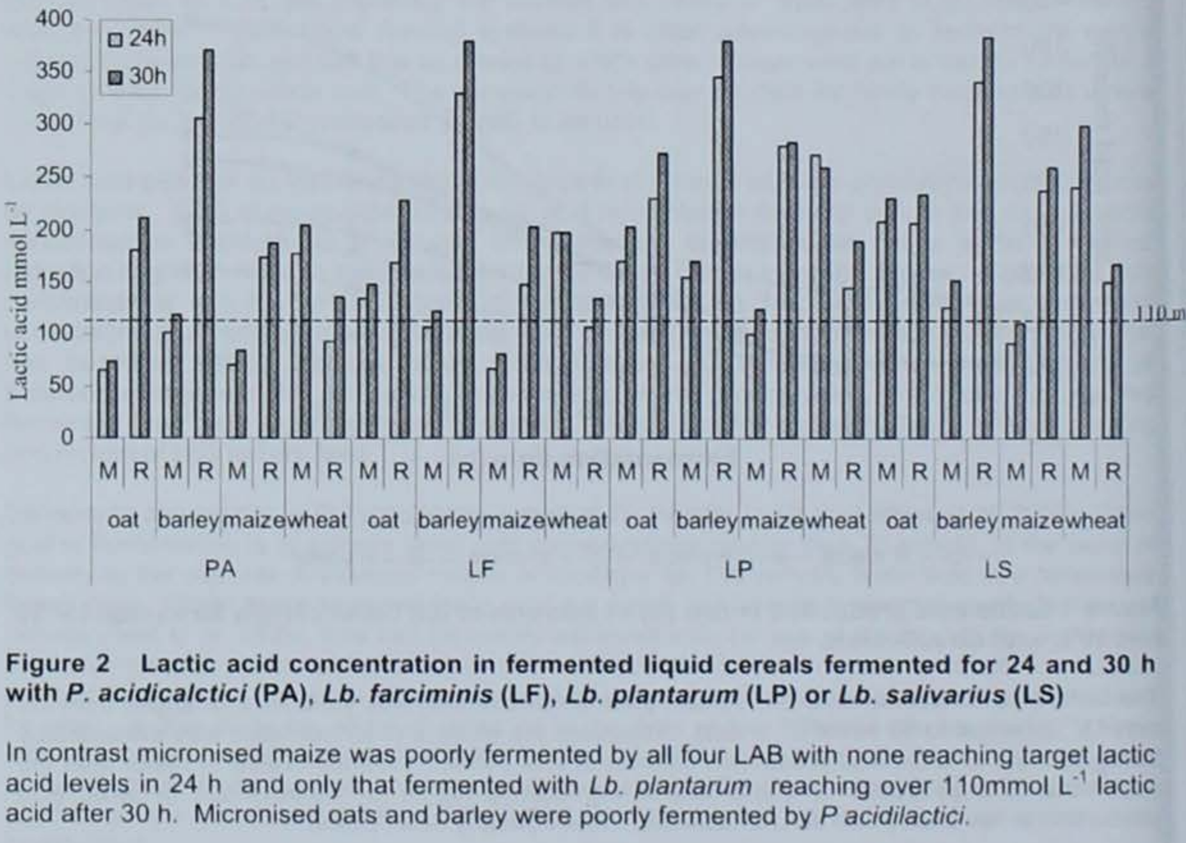

Figure 2 Lactic acid concentration in fermented liquid cereals fermented for 24 and 30 h with *P. acidicalctici* (PA), *Lb. farciminis* (LF), *Lb. plantarum* (LP) or *Lb. salivarius* (LS)

In contrast micronised maize was poorly fermented by all four LAB with none reaching target lactic acid levels in 24 h and only that fermented with *Lb. plantarum* reaching over 110mmol L^{-1} lactic acid after 30 h. Micronised oats and barley were poorly fermented by *P acidilactici*.

Discussion

The results of this study demonstrated that cereals are not equally well fermented by lactic acid bacteria. Apart form wheat, raw cereals were fermented to a greater degree than micronised cereals. This was possibly due to breakdown of complex carbohydrate in the grains to simpler oligosaccharides and sugars by endogenous enzyme action. These simpler sugars would be fermented more efficiently by the lactic acid bacteria. The process of micronisation destroys endogenous enzymes and breakdown of complex carbohydrate would be limited to that of microbial enzyme action. This was reflected in reduced rates of lactic acid production in barley, maize and to a large extent oats. The picture with wheat was the opposite, with all four organisms fermenting micronised wheat much more efficiently than raw wheat. The reasons for this are unclear and further investigations are being undertaken.

All four organisms produced by far the most lactic acid in raw barley, *ca* 100 mmol L^{-1} in excess of any other substrate. The reasons for this are unclear but it may be that the endogenous enzymes n barley efficiently breakdown storage polysaccharides and release simple sugars such as glucose and fructose for use by LAB.

In practice micronised cereals are only used to formulate diets for newly weaned pigs and the practice of fermenting feed for very young pigs is limited to a small number of producers. However, the results here suggest that producers following this practice would achieve the best results in terms of feed stability and safety if the majority of the cereal in the feed was wheat. Most producers practicing fermentation use raw cereals. In this case by far the best results in terms of feed stability and safety would be produced with barley. If fermented barley containing lactic acid levels of 350 mmol L^{-1} is used to make up a complete feed then that feed would contain approximately 240 mmL^{-1} lactic acid at the point of delivery to the pig. It is unlikely that this level of lactic acid would affect feed intake, but it would be sufficient to prevent the proliferation of any enteropathogenic organism that might contaminate the feed in the trough.

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References

BEAL, J. D., NIVEN, S. J., BROOKS, P. H. and GILL, B. P. 2005. Variation in short chain fatty acid and ethanol concentration resulting from the natural fermentation of wheat and barley for inclusion in liquid diets for pigs. *Journal of the Science of Food and Agriculture*, 85, (3) 433-440.

BEAL, J. D., NIVEN, S. J., CAMPBELL, A. and BROOKS, P. H. 2002. The effect of temperature on the growth and persistence of salmonella in fermented liquid pig feed. *International Journal of Food Microbiology*, 79, (1-2) 99-104.

NIVEN, S. J., BEAL, J. D. and BROOKS, P. H. 2004. The simultaneous determination of short chain fatty acid, monosaccharides and ethanol in fermented liquid pig diets. *Animal Feed Science and Technology*, 117, (3-4) 339-345.

VAN WINSEN, R. L., URLINGS, B. A. P., LIPMAN, L. J. A., SNIJDERS, J. M. A., KEUZENKAMP, D., VERHEIJDEN, J. H. M. and VAN KNAPEN, F. 2001. Effect of fermented feed on the microbial population of the gastrointestinal tracts of pigs. *Applied and Environmental Microbiology*, 67, (7) 3071-3076.